Remarks

I. The Office Action

Claims 28-38, 43, 44, 47, and 48 are rejected under 35 U.S.C. § 103(a) for allegedly being obvious over U.S. Patent 4,792,447 ("the Uhr patent") in view of International Patent Publication WO 03/004056 ("the Raison publication"), Stavnezer et al., *J. Immunol., 137*, 3978-3982 (1986) ("the Stavenzer article") and Abe et al., *Am. J. Clin. Path., 100*, 67-74 (1993) ("the Abe article"). The examiner also rejects claims 39-42, 45, and 46 under 35 U.S.C. § 103(a) for allegedly being obvious in view of the Uhr patent in view of the Raison publication and the Abe article, and in further view of U.S. Patent Publication No. US 2005/0255532 ("the Ruben publication"). Reconsideration of the rejections is respectfully requested.

II. Amendments to the Claims

Claims 28, 33 and 40 have been amended to clarify that the lymphoid target cells of the recited methods are "lambda-type" lymphoid cells, *i.e.*, those that express free lambda light chain (referred to as LMA in the present application) rather than free kappa light chain on their surface. Malignant B cells producing antibodies comprising the two different light chain subtypes have been associated with specific clinical or biologic presenting features, such as bone involvement and renal impairment, and with different clinical outcome in patients. See, for example, Magrangeas *et al., Blood, 101*(12): 4998-5006 (2003) (Exhibit A hereto). The amendment is supported by the specification at, for example, page 5, lines 25-29 and page 13, lines 31-35. No new matter is introduced by the amendments.

III. The Rejection of Claims 28-38, 43, 44, 47 and 48 Under Section 103(a) Should Be Withdrawn

The rejection that it would have been obvious to one of ordinary skill in the art to create the claimed subject matter is based on the assertions that: the Uhr patent teaches an antibody conjugate that binds lambda light chain on tumor cells and is used to treat B cell tumors, the Raison publication teaches that malignant B cells in multiple myeloma patients can produce kappa or lambda light chains, the Stavnezer article teaches that free lambda light chain can be expressed on the surface of a leukemia cell and the Abe article teaches

antibodies that bind free lambda light chain but not immunoglobulin-associated lambda light chain.

In response, Applicants respectfully submit that the none of the cited documents alone or in combination suggests the methods of claims 28-38 or the products of claims 43, 44, 47 and 48. The pending claims are directed, at least in part, to methods for the treatment or prophylaxis of a B-cell disorder in a subject or inhibiting the growth or killing lymphoid cells in a subject. The methods comprise administering to the subject an anti-LMA antibody or an LMA ligand to inhibit the growth of, or kill, lambda-type lymphoid cells in the subject (claims 28 and 33). The pending claims also provide a method for autologous hematopoietic cell transplantation in a subject, the method comprising removing a hematopoietic progenitor cell population from the subject, treating the cell population with an anti-LMA antibody or LMA ligand conjugate, and transplanting the treated cell population into the subject (claim 38). The anti-LMA antibody and LMA ligand specifically bind LMA and do not bind lambda light chain associated with an immunoglobulin heavy chain.

None of the cited documents suggest the concept of targeting LMA (free lambda light chain) on the surface of lambda-type lymphoid cells to treat B cell disorders.

The examiner acknowledges that the Uhr patent does not teach treating multiple myeloma and that it does not teach its antibody binds free lambda light chain. While the examiner refers to the first full paragraph column 4 of the Uhr patent as supposedly disclosing antibody that binds lambda light chain, that paragraph of the Uhr patent actually only discusses antibodies that bind lambda light chain existing in an intact antibody molecule, that is lambda light chain in association with antibody heavy chain. It states "[a]ntibodies can be developed which are specific for the collective class of immunoglobulins by employing the specificity for the λ or κ light chain portion of an immunoglobulin, which light chains are common to all classes of immunoglobulins" (emphasis added). Moreover, the summary, examples and claims of the Uhr patent all relate to antibodies that bind intact immunoglobulin, most particularly IgD immunoglobulin. In contrast, the present claims specify that the recited antibody does not bind lambda light chain in association with antibody heavy chain. Moreover, the Uhr patent does not suggest specifically targeting

lambda-type lymphoid cells that express free lambda light chain on their surface. Thus, the Uhr patent does not suggest the subject matter of the present claims.

The combination of the secondary references with the Uhr patent does not cure the deficiencies of the Uhr patent.

The Raison publication relates to antibodies specific for an antigen expressed on the surface of kappa-type myeloma cells designated kappa myeloma antigen. Kappa myeloma antigen is disclosed to consist of free kappa light chains expressed in non-covalent association with actin on the cell membrane. In support of the rejection, the examiner relies on the statement in the paragraph at lines 29-33 of the first page of the Raison publication that "malignant B-cells in MM produce excess of amounts of light chain" but the examiner appears to disregard the remaining part of the statement that the "light chains are present in the serum and urine of individuals." This is in contrast to the methods of the present claims which based on targeting free lambda light chain on the surface of cells.

The examiner also argues that it would have been obvious that malignant cells would express free light lambda light chains on their surface since the Raison publication discloses cells expressing free kappa light chains on their surface.

As part of the response to the previous Office Action, Applicants submitted a Declaration under 37 C.F.R. § 1.132 of Cameron Jennings, Ph.D., setting out various structural, functional and expression differences between lambda and kappa light chains. Applicants continue to rely on the evidence set out in the declaration to show it would not have been predictable that there would be free lambda light chain localized on the surface of B cells in disorders and hence, it would not have been obvious to target free lambda light chains on the surface of lambda-type lymphoid cells from the combination of documents cited by the examiner.

First, there is nothing inherent in the structure of lambda light chains that suggests that the proteins are incorporated into cell membranes. (See Rule 132 Declaration at paragraph 17.) Light chains are composed, in part, of seven beta strands that form a sandwich of two beta sheets. This structural motif is found on a vast array of protein sub-

families with diverse biological activities and sub-cellular locations. Kappa and lambda light chains do not contain structural motifs that would suggest that free light chains are localized to the cell membrane, there is no membrane targeting sequence found in light chain proteins, and no normal biological function has been attributed to light chains alone (i.e., in the absence of immunoglobulin heavy chains). (See Rule 132 Declaration at paragraph 17.)

Further, the membrane interaction of kappa light chains occurs via hydrophobic and/or electrostatic interactions, and kappa light chain expression on B-cells is not predictive of free lambda light chain localization due to differences in lambda light chain structure that mediate hydrophobic and/or electrostatic interactions. (See Rule 132 Declaration at paragraph 21.) As explained by Dr. Jennings in the Rule 132 declaration, the kappa and lambda genes differ in the number and general arrangement of variable (V), joining (J) and constant (C) genes. (See Rule 132 Declaration at paragraph 14.) For example, there is a single C kappa gene while there are five functional C lambda genes. (*Id.*) In addition to comprising different arrangements of V, J, and C domains, the amino acid sequences *within* the regions differ. Kappa and lambda proteins share minimal sequence identity within their C domains. (*Id.* at paragraph 15.) Additionally, genetic recombination events and somatic hypermutation results in variability in the V domains of kappa and lambda light chains. (*Id.*) The differences in the primary structure of kappa and lambda light chains is significant, as highlighted by the divergence in processes used to force "strand switching" in kappa and lambda light chains. (*Id.* at paragraph 16.) The "strand switch" phenomenon is dependent on local structure, and confirms the variability in sequence, structure, and biological function between the two immunoglobulin light chains. (*Id.*) The distinct structure of kappa and lambda light chains is further evinced by the selective binding of the *Peptostreptococcus magnus* protein L to kappa light chains and *not* lambda light chains. (*Id.* at paragraph 18.)

The differences in primary structure of free kappa and lambda light chains affect not only the charge of the exposed side chains of the proteins, but also the proteins' tendency to form monomers (kappa light chains) or dimers (lambda light chains). The proteins' structural differences yield different pathological conditions. (*Id.* at paragraph 19.) Lambda antibodies are found in two-thirds of light chain amyloidosis (AL amyloidosis) cases, whereas kappa light chains mediate greater than 85% of Light-Chain Deposition Disease (LCDD), a distinct protein deposition disease. (*Id.*) The character and rate of the catabolic processes involved in

the clearance of kappa and lambda light chains are different, and the difference in the structure of amyloid fibrils (fibrilar) and the LCDD deposits (amorphous) further reflects the difference in the general structure of kappa and lambda light chains. (*Id.* at paragraph 20).

Because of these distinctions between kappa and lambda light chains, the expression of kappa light chains on the B cell surface as described in the Raison publication does not predictably suggest that free lambda light chain is localized on the surface of, *e.g.*, some myeloma cells. (*Id.* at paragraphs 22 and 23.) The amendments to the claims specifying that the cells targeted are lambda-type lymphoid cells highlights the difference between the disclosure of the documents cited by the examiner and the claimed subject matter.

The examiner cited the Stavnezer article in response to the Rule 132 declaration, arguing that the article shows the expression of free lambda light chain on the surface of B cells. The Stavnezer article though relates to the HL-60 cell line (established from a patient with acute promyelocytic leukemia) which comes from a different cell lineage than the cells of the claims. There are two main types of progenitors of blood cells, the lymphoid progenitor cell and the myeloid progenitor cell. B cells develop from a lymphoid progenitor cell while HL-60 cells developed from a myeloid progenitor cell. B cells and promyelocytes have different characteristics and functions. Expression of free lambda light chain on the surface of HL-60 cells would not have predicted expression of free lambda light chain on the surface of lymphoid cells.

The examiner also relies on the Abe article disclosure of antibodies that bind free lambda light chain found in serum or urine but do not bind immunoglobulin-associated lambda light chain. The disclosure of the existence of antibodies against free lambda light chain found in serum or urine does not suggest free lambda light chain on the surface of lambda-type lymphoid cells as a target for the destruction of the cells.

Thus, the Uhr patent, the Raison publication, the Stavnezer article and the Abe article, alone or in any combination, fail to render obvious a method for treating a B-cell disorder, inhibiting or killing lambda-type lymphoid cells, or treating a cell population for autologous hematopoietic cell transplantation by targeting free lambda light chain on the surface of the

lambda-type cells. Likewise, the cited documents do not render obvious the anti-LMA antibody of claims 43 and 47 or the pharmaceutical composition of claim 48.

IV. The Rejection of Claims 39-42, 45 and 46 Under Section 103(a) Should Be Withdrawn

In the second rejection directed to the claimed autologous hematopoietic cell transplantation and lymphoid cell localization subject matter, the examiner again cites the Uhr patent, the Raison publication, and the Abe article but also adds the Ruben publication to the combination. The examiner contends that the Rubin publication discloses therapeutic use of chimeric antibodies, diagnostic use of labeled anti-tumor antibodies, and antibody conjugates, and that "[a] routineer would have treated the autologous cell transplant recipient with the anti-lambda antibody to kill tumor cells present in the recipient."

The Rubin publication does not relate to lambda light chain or any protein expressed on lymphoid cells but instead relates to antibodies that bind a member of the tumor necrosis superfamily termed "B Lymphocyte Stimulator" or "BLyS." BLyS is expressed on cells of myeloid origin, not on lymphoid cells. See Rubin paragraph [0004]. Thus, the Rubin publication, like the Uhr, Raison, and Abe documents explained above, fails to teach or suggest targeting free lambda light chain on the surface of lambda-type lymphoid cells. The second combination of documents therefore also fails to render obvious the subject matter of claims 39-42, 45 and 46.

V. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398 (2007)

The examiner raised *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398 (2007) in support of the Section 103 rejections. Applicants submit that under the standard articulated by the Supreme Court the subject matter of the claims is not obvious as explained above because no combination of the cited documents suggested methods or products targeting free lambda light chain on the surface of lambda-type lymphoid cells to treat or detect/diagnose B cell disorders.

The rejections under Section 103 may properly be withdrawn.

Conclusion

Applicants submit the claims are in good and proper form for allowance, and the examiner is respectfully requested to pass this application to issue.

This paper is accompanied by petition for a one-month extension of time with the required fee. The commissioner is authorized to charge any additional fees due in connection with this filing to Marshall, Gerstein and Borun, LLP deposit account number 13-2855, under order no. 29729/38914.

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Respectfully submitted,
Electronic signature: /Greta E. Noland, Reg. #35,302/
Greta E. Noland
Registration No.: 35,302
MARSHALL, GERSTEIN & BORUN LLP
233 S. Wacker Drive, Suite 6300
Sears Tower
Chicago, Illinois 60606-6357
(312) 474-6300
Agent for Applicants

Gene expression profiling of multiple myeloma reveals molecular portraits in relation to the pathogenesis of the disease

Florence Magrangeas, Valéry Nasser, Hervé Avet-Loiseau, Béatrice Loriod, Olivier Decaux, Samuel Granjeaud, François Bertucci, Daniel Birnbaum, Catherine Nguyen, Jean-Luc Harousseau, Régis Bataille, Rémi Houlgatte, and Stéphane Minvielle

Although multiple myeloma (MM) is a unique entity, a marked heterogeneity is actually observed among the patients, which has been first related to immunoglobulin (Ig) types and light chain subtypes and more recently to chromosomal abnormalities. To further investigate this genetic heterogeneity, we analyzed gene expression profiles of 92 primary tumors according to their Ig types and light chain subtypes with DNA microarrays. Several clusters of genes involved in various biologic functions such as immune response, cell cycle control, signaling, apoptosis, cell adhesion, and structure significantly discriminated IgA- from IgG-MM. Genes associated with inhibition of differentiation and apoptosis induction were up-regulated while genes associated with immune response, cell cycle control, and apoptosis were down-regulated in IgA-MM. According to the expression of the 61 most discriminating genes, BJ-MM represented a separate subgroup that did not express either the genes characteristic of IgG-MM or those of IgA-MM at a high level. This suggests that transcriptional programs associated to the switch could be maintained up to plasma cell differentiation. Several genes whose products are known to stimulate bone remodeling discriminate between κ- and λ-MM. One of these genes, *Mip-1α*, was overexpressed in the κ subgroup. In addition, we established a strong association ($P = .0001$) between κ subgroup expressing high levels of *Mip-1α* and active myeloma bone disease. This study shows that DNA microarrays enable us to perform a molecular dissection of the bioclinical diversity of MM and provide new molecular tools to investigate the pathogenesis of malignant plasma cells. (Blood. 2003;101:4998-5006)

Introduction

Multiple myeloma (MM) is characterized by the accumulation of malignant plasma cells (PCs), usually within the bone marrow. Besides the demonstration of this excess of PCs, the diagnosis of MM is usually supported by the finding of lytic bone lesions on x-rays and the presence of a monoclonal immunoglobulin (Ig) in the serum and/or urine. The monoclonal Ig allows us to define several types of MM, depending on the Ig heavy chain (IgH) isotype and light chain subtype (IgL). Most MMs are characterized by the excretion of a complete monoclonal Ig, easily detectable on the serum electrophoresis. The most frequent Ig is IgG (about 60% of the patients), followed by IgA (about 25%). In a few cases, other Ig classes are observed—that is, IgD, IgM, or IgE (less than 2% of the patients). In other instances, malignant PCs do not excrete any Ig chain, representing about 1% of the patients. Finally, approximately 15% of the patients excrete only light chains: the so-called Bence Jones MM (BJ-MM). Apart from these IgH characteristics, the M component may be further classified upon the light chain subtype—that is, either the κ chain or the λ chain. Roughly two thirds of the patients present a κ-type MM and one third a λ-type MM, a proportion similar to that observed in normal PCs. These light chains are often produced in excess, and even in common IgG- or IgA-MM, free light chains are detected in the serum and/or urine.

Of note, these different Ig types and light chain subtypes have been associated with specific clinical or biologic presenting features, such as bone involvement and renal impairment, and with different clinical outcome.[1] However, so far, no published study has addressed the question of possible different biologic behaviors in these different types of MMs—that is, IgG- versus IgA-MM, IgG- and IgA-MM versus BJ-MM, or κ-type versus λ-type MM. The recent development of the microarray technology has opened new windows on the way to approach specific intracellular biologic pathways[2,3] and to improve tumor classifications.[4-9] To address this issue, we have analyzed a large series of patients with MM using gene expression profiling, focusing our analysis on the differences in gene activation (or repression) associated with the different Ig types—that is, IgG versus IgA, and κ light chain versus λ light chain subtypes.

Patients, materials, and methods

Patients

The diagnosis of MM was done according to the criteria of the Southwest Oncology Group.[10] From a total of 105 patients analyzed in the present study, high-quality gene expression data were obtained on 88 MMs and 4

From INSERM U463, Department of Clinical Hematology, University Hospital, Nantes, France; INSERM ERM206; and INSERM U119, Institut Paoli-Calmettes, Marseille, France.

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S.M. and R.H. equally supervised this work.

Reprints: Stéphane Minvielle, INSERM U463, Institut de Biologie, 9 Quai Moncousu, 44095 Nantes Cedex 1, France; e-mail: sminviel@nantes.inserm.fr; and Rémi Houlgatte, ERM206 INSERM, Parc Scientifique de Luminy, case 906, 13288 Marseille Cedex 9, France; e-mail: houlgatte@tagc.univ-mrs.fr.

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primary cell leukemia (PCLs) defined by more than 20% of malignant PCs in the peripheral blood. Newly diagnosed untreated patients were referred to one of the clinical centers of the Intergroupe Francophone du Myélome (IFM). Written informed consent was obtained from all the patients according to the Declaration of Helsinki. The median age of the patients was 60 years (range, 31-78 years). The clinical staging was established according to standard criteria of Durie and Salmon[11]: 14 stage I, 7 stage II, 67 stage III, and 4 PCL. The monoclonal Ig was IgGκ in 28 patients, IgGλ in 22 patients, IgAκ in 16 patients, IgAλ in 6 patients, BJPκ in 12 patients, and BJPλ in 8 patients. Correlations of the 2 strongest prognostic factors, β_2-microglobulin (β_2M) and chromosome 13 abnormalities, across the isotypes and subtypes subgroups were analyzed. Serum β_2M levels and chromosome 13 deletions (fluorescent in situ hybridization analysis) were not significantly different among these MM subgroups.

PC purification and total RNA isolation

Mononuclear bone marrow cells were separated using gradient density centrifugation (lymphocyte separation medium, Eurobio, Les Ulis, France), and plasmacytosis was evaluated by morphology in these mononuclear cell suspensions. PCs were then positively selected using anti-CD138–coated microbeads (Miltenyi, Paris, France), because CD138 is expressed only on PCs (normal and malignant) within the bone marrow. Purity and viability of the positively selected cell suspension was assessed by morphology and was above 96% in all the cases. One million PCs were used to prepare total RNA using the guanidinium thiocyanate–phenol method.[12] The RNA integrity was randomly verified by using an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA). Half the preparation was used to generate a complex probe.

Gene expression profiling procedures

Vector oligomer labeling and hybridization conditions. One microgram of vector oligonucleotide was labeled at the 5′ end with 30 μCi (1.11 MBq) [γ-^{33}P]adenosine triphosphate ([γ-^{33}P]ATP) and 10 units of T4 polynucleotide kinase (Invitrogen, Cergy Pontoise, France) for 45 minutes at 37°C; unincorporated nucleotides were removed by purification on a Sephadex G-25 column (Roche, Meylan, France). The vector oligonucleotide sequence used was 5′-ACTGGCCGTCGTTTTACA. Nylon microarrays were prehybridized in hybridization mix (5 × SSC, 5 × Denhardt solution, 0.5% sodium dodecyl sulfate [SDS]) for 4 hours at 42°C and then hybridized with the vector probe for 2 hours at 42°C. After hybridization, filters were washed in 2 × SSC, 0.1% SDS at room temperature for 10 minutes and at 42°C for 5 minutes. After phosphor screen acquisition (Fuji BAS 5000; Fuji, Tokyo, Japan), filters were stripped in 0.1 × SSC, 0.1% SDS at 68°C for 3 hours before hybridization with a complex probe. Quantification of the vector probe hybridization signal provided a value corresponding to the amount of DNA fixed in each spot of the microarray.

Preparation and labeling of complex probes from total RNA and hybridization conditions. Aliquots of 2.5 μg total RNA, 8 μg oligo(dT)$_{25}$ to saturate long polyA tails, and 0.3 ng in vitro–synthesized *Arabidopsis thaliana* cytochrome c554 allowing interfilter normalization were mixed, heated to 70°C for 8 minutes, and cooled to 42°C before reverse transcription in a reaction mixture containing 50 mM Tris (tris(hydroxymethyl)aminomethane)–HCl (pH 8.3), 75 mM KCl, 10 mM dithiothreitol (DTT), 3 mM $MgCl_2$, 5 units RNase inhibitor (GIBCO BRL, Cergy Pontoise, France), 0.4 mM each of deoxyuridine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP), 240 nM deoxycytidine triphosphate (dCTP), 30 μCi (1.11 MBq) [α-^{33}P]dCTP, and 400 units Superscript RNAse H reverse transcriptase (GIBCO BRL) for 2 hours at 42°C. After alkali treatment and neutralization, unincorporated nucleotides were removed by purification on a Sephadex G-50 column (Roche). The complex probe was then incubated for 2 hours at 65°C with 2 μg poly(dA)$_{80}$ to eliminate spurious hybridization via the polyA tail present in some clones before hybridization. Nylon microarrays were prehybridized in 1 mL hybridization mix for 6 hours at 68°C and then hybridized with the complex probe in 0.4 mL hybridization mix for 48 hours at 68°C. After hybridization, filters were washed twice in 0.1 × SSC, 0.1% SDS at 68°C for 90 minutes.

Nylon microarray technology. The feasibility, reproducibility, and sensitivity of spotting procedures onto nylon membrane currently used in our laboratory to produce cDNA arrays have been previously described.[13-16]

All cDNA clones were chosen using the expressed sequence tag (EST) database from the NCBI: http://www.ncbi.nlm.nih.gov. The clones were selected from libraries constructed with cloning vectors pT7T3D or Lafmid BA, same host bacteria, and an insert size of approximately 1 kb. Clones were provided by the Human Genome Mapping Project Resource Centre (Hinxton, United Kingdom). From our 7200 cDNA library, preferentially composed of genes expressed in carcinogenesis and immune response, we used 5376 cDNA clones to design our nylon microarrays. The selection of these clones was based on preliminary DNA microarray expression data obtained with a cDNA probe prepared from a pool of total RNA isolated from 4 different MM cell lines and 2 MM patients hybridized to a microarray containing 7200 cDNAs.

The cDNA clones were amplified in 96-well microtiter plates with 5′-GTGGAATTGTGAGCGGATAAC and 5′-GCAAGGCGATTAAGTTGGG. Polymerase chain reaction (PCR) products with more than one band or with an unexpected size were rejected. PCR product concentration was adjusted to 0.3 μg/μL. PCR products were spotted onto Hybond N+ filter (Amersham, Orsay, France) using GMS 417 arrayer. DNA spotted was then denatured and UV–cross-linked onto nylon filter. All membranes contained a set of control spots. The pT7T3D vector, poly(dA)$_{80}$, vector oligonucleotide, and 50 PCR reactions without template were used as negative controls. Many genes were spotted in duplicate spots to assess the reproducibility. *Arabidopsis thaliana* cytochrome c554 clone that is devoid of similarities to human DNA sequences is used to normalize the differences in labeling of each complex probe.

The reproducibility of complex probe hybridization was verified first by analyzing a variation in average intensity difference. Up to 3% of the clones showed a 2-fold difference in signal intensity.[15] Secondly, we compared 2 microarrays hybridized with complex probes prepared with 2 RNA samples extracted separately from the same patient. The samples were always found clustered in directly adjacent columns. We have demonstrated previously that the hybridization signal is proportional to the abundance of individual species in the complex probe and to the amount of PCR products spotted onto the microarray. In our hybridization conditions the minimum sample for detection is 0.2×10^6 molecules. In addition, the amount of PCR products per spot being 1500 times more than the detection limit, the signal measured for highly expressed genes is not saturated.

Sample quality standard. In this study we obtained high-quality gene expression data on 92 of 105 samples (88%). The quality is estimated by measuring the signal intensity of all clones in a microarray and the number of spots detected; if these 2 parameters are too low the microarray is not considered for the study.

Data acquisition and normalization. DNA microarrays were scanned at 25-μm resolution in an image plate system (Fuji BAS 5000; Fuji). The hybridization signals were quantified using ArrayGauge software v.1.3 (Fuji). A background value for each membrane hybridization was calculated from negative controls and subtracted to each expression value. The data were corrected for the amount of PCR product detected by the vector probe and normalized using *Arabidopsis thaliana* cytochrome c554 control clone. Sets of genes that were not measured on at least 60 of the 92 samples were removed. Statistical analyses were performed using the remaining set of 2600 genes.

Statistical analysis

Samples and genes were median centered and log transformed before data analysis. Gene and sample classifications were obtained by unsupervised hierarchic clustering using uncentered correlation distance and average linkage aggregative method with the Cluster and Tree View softwares (M. Eisen, http://www.microarrays.org/software).[17]

Gene-discriminating particular subgroups of MM were searched using a signal-to-noise calculation: $DS = (\mu 1 - \mu 2)/(\sigma 1 + \sigma 2)$; where $\mu 1$ and $\sigma 1$, respectively, represent mean and standard deviation of the expression levels of the gene in subgroup 1, and $\mu 2$ and $\sigma 2$ represent mean and standard deviation of the same gene in subgroup 2.[4] A total of 200 random permutations of the samples were used to calculate significance level at

1:10 000 risk, giving less than 1 gene found by chance (false-positive gene). The data sets used for the identification of genes that distinguish Ig subtypes or isotypes are available at our website: http://tagc.univ-mrs.fr/pub/.

RT-PCR procedures

The first cDNA strand was synthesized using total RNA (2.5 μg) at 37°C for 1 hour in a 50-μL reaction mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$, 5 units RNase inhibitor (GIBCO BRL), 0.5 mM of each deoxynucleotide triphosphate (dNTP), 400 units Moloney murine leukemia virus (M-MLV) reverse transcriptase (Gibco), and 0.5 μg oligo(dT) 15 mer. Five microliters of the reaction mixture was made up to 50 μL using Taq polymerase buffer (10 mM Tris-HCl [pH 9], 50 mM KCl, 1.5 mM $MgCl_2$) containing 25 pmol of each primer, 1 mM of each deoxynucleotide triphosphate (dNTP), and 1 unit of AmpliTaq DNA polymerase (Amersham). Amplifications were performed using a thermal cycler for 20 cycles under the following conditions: denaturation for 1 minute at 94°C, annealing for 1 minute at 55°C, and elongation for 1 minute at 72°C. *Mip-1α* was amplified using the following primers: sense primer: 5′-CGAGCCCACATTCCGTCACC-3′ and antisense primer: 5′-CCATGACTGCCTACACAGGC-3′. PCR products were separated in a 1% agarose gel and directly visualized after ethidium-bromide staining. Expression of β-actin using primers 5′-ATCTGGACCACACCTTCTACAATGAGCTGCG-3′ and antisense 5′-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′ was assessed to ensure uniformity of amplification.[18]

Results

In this study, we determined the gene expression profiles of 88 newly diagnosed MMs and 4 primary PCLs according to their Ig types and light chain subtypes because M component is the major source of biologic heterogeneity in MM patients.

From highly enriched $CD138^+$ cells of each tumor sample, 2.5 μg total RNA was extracted and used to prepare radioactively labeled cDNA complex targets. Hybridizations were carried out on DNA microarrays containing 5376 genes. Radioactive dot intensities of scanned images were measured and normalized to yield a ratio of background-corrected single-dot intensity to background-corrected median-dot intensity (see "Patients, materials, and methods"). The study was performed using a set of 2600 genes that were significantly expressed across the MM patients. In the initial analysis of the gene expression data, we applied an unsupervised hierarchic clustering algorithm to group the myeloma samples on the basis of similarities in their expression of these genes. The same clustering method was used to group genes on the basis of similarity in their pattern of expression over all the samples. This analysis revealed that the patients were not grouped according their Ig light chain subtypes or Ig heavy chain isotypes, and genes encoding Ig were found in 4 highly contrasted clusters (Figure 1). We next analyzed gene expression profiles of the patients by using a discriminating score (DS) based on signal-to-ratio calculation[4] to identify and rank the differentially expressed genes among biologic subtypes of MM. The higher score denotes the greater ability to differentiate the 2 MM groups. We used a random permutation test, patients were randomly permutated (200 times) into 2 groups, and for each gene a DS was calculated. A gene significantly distinguishes the 2 groups of patients if it passed a 99.99% significance threshold (α less than 0.0001).

First, we compared expression data of one subgroup including 45 IgG-MMs and 3 IgG-PCLs with a second subgroup including 21 IgA-MMs. The DS test yielded 61 unique cDNA sequences from 58 different genes whose change in expression among all the patients best distinguished IgG-MM from IgA-MM (Table 1). Hierarchic cluster analysis of the 69 MM samples was performed

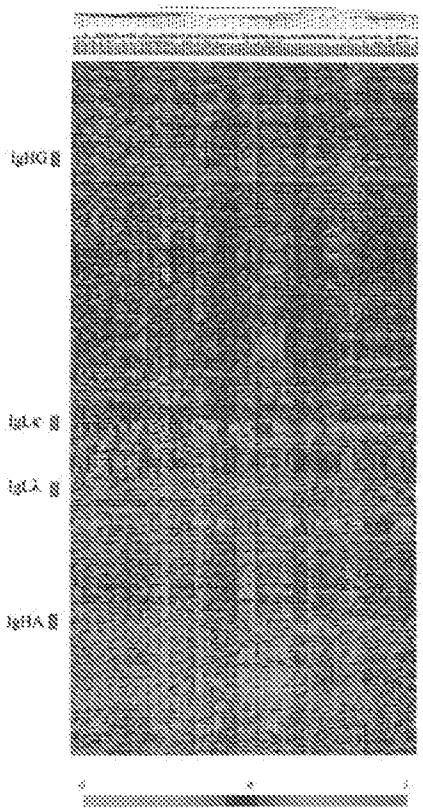

Figure 1. Hierarchic clustering of 92 diagnostic MM and PCL samples (columns) versus 2600 genes (rows). The normalized expression value for each gene is depicted according to the scale at the bottom; red indicates expression levels greater than the median, and green indicates levels less than the median (−2 to 2 in log base 2 units). Gray indicates excluded values.

using expression data of the 61 selected genes; the algorithm perfectly segregated IgG-MM from IgA-MM (data not shown). Figure 2 shows the matrix depicting gene expression values of the MM samples with rows representing characterized genes (49 elements) grouped according to putative biologic functions including immune response, cell cycle control, Notch signaling, cell adhesion and structure, and apoptosis. Most of these genes were up-regulated in IgG-MM versus IgA-MM. The highest differentiating score, except for IgH genes, was found for *GATA6*, a member of the GATA transcription factor family, which has recently been shown to regulate a WNT family member, WNT7b.[19] Immune response–associated genes were up-regulated in IgG-MM (eg, the cytokines *IL-1β* and *IL-16*, the cytokine receptors *CSF-1R* and CSF-3R, and the B-cell development regulators *Id2* and *CSK*). Three transcription factors (*BRCA1*, *ZNF148*, *c-myb*) involved in cell cycle control were also up-regulated in IgG-MM, whereas 2 genes involved in Notch signaling in hematopoietic cells (*Jagged2*, *GATA2*) were down-regulated in this subgroup of MM. Among the 6 genes potentially associated with cell adhesion and structure, 2 integrins (*ITGA1* and *ITGA2*) as well as *ACTB* were up-regulated in IgG-MM, whereas *AP1B1* and *DCTN1* were significantly up-regulated in IgA-MM. Only 2 members of the apoptosis class were significantly differentially expressed in these subgroups of MM. The first gene, *DAP-1*, a proapoptotic factor, was overexpressed in IgG-MM, while the second, *SRF*, a regulator of the antiapoptotic molecule *Mcl-1*, was down-regulated in the same subgroup of MM.

Second, we used the 61 genes that best discriminated IgG- and IgA-MM to investigate whether BJ-MM could represent a distinct group or not. Using hierarchic clustering algorithm, MM samples

Table 1. Statistically different genes between IgG-MM and IgA-MM

Gene symbol	Gene description	Accession no.	Discriminating score
IGHG3	IgG	H88233	−2.75
GATA6	GATA-binding protein 6	N91601	−2.39
IGHG3	IgG	N92646	−2.07
IGHG3	IgG	H64493	−2.04
HADHB	Hydroxyacyl-CoA dehydrogenase, beta subunit	T68767	−1.89
IF	I factor (complement)	H89710	−1.29
BRCA1	Breast cancer 1, early onset	H90415	−1.29
ESTs	Unknown	W46567	−1.10
CSF-1R	Colony-stimulating factor 1 receptor, v-fms oncogene homolog	H57126	−1.00
ESTs	Unknown	H93533	−0.98
ITGA1	Integrin, alpha 1	H68922	−0.88
FBG	Fibrinogen, B beta polypeptide	H91714	−0.86
Id2	Inhibitor of DNA binding 2, dominant-negative helix-loop-helix	H82442	−0.85
IL-16	Interleukin-16 (lymphocyte chemoattractant factor)	H57532	−0.84
IL-1B	Interleukin-1, beta	W47101	−0.80
ZNF148	Zinc finger protein 148 (pHZ-52)	H70711	−0.79
KLF1	Kruppel-like factor 1 (erythroid)	H60702	−0.76
ITGA2B	Integrin, alpha 2b (antigen CD41B)	AA905458	−0.71
ESTs	Unknown	AA279804	−0.71
CSK	c-src tyrosine kinase	H90752	−0.70
ESTs	Unknown	H22578	−0.70
PNUTL1	Peanut (*Drosophila*)-like 1	AA702163	−0.65
ALAS2	Aminolevulinate, delta-, synthase 2	N54577	−0.63
PSMB7	Proteasome subunit, beta type, 7 (large multifunctional protease 7)	N52556	−0.61
ESTs	Unknown	N52646	−0.61
DAP-I	Death-associated protein 1	H65452	−0.59
FBP1	Fructose-1,6-bisphosphatase 1	N57706	−0.58
DEHUE	Glutamate dehydrogenase	N57779	−0.58
ACTB	Actin beta	H54441	−0.58
CSF-3R	CSF3R: colony-stimulating factor 3 receptor (granulocyte)	R31950	−0.56
TBPG	Trophoblaste glycoprotein	R70262	−0.56
APOH	Apolipoprotein H	R06430	−0.54
MYB	v-myb avian myeloblastosis viral oncogene homolog	N49284	−0.53
NGRFAP1	Nerve growth factor receptor (TNFRSF16)-associated protein 1	W68632	0.54
CST3	Cystatin C	W93398	0.54
DARS	Aspartyl t-RNA synthetase	H28873	0.55
ESTs	Unknown	AA626888	0.56
PSG2	Pregnancy-specific beta-1–glycoprotein 2	R65579	0.57
COL 16A1	Collagen type XVI alpha 1	R54968	0.57
SRF	Serum response factor (c-fos SRE-binding transcription factor)	W32378	0.57
ABCA7	ATP-binding cassette, subfamily A, member 7	H45443	0.60
ESTs	Unknown	H51280	0.61
ARD1	N acetyltransferase	R55220	0.65
FALZ	Fetal Alzheimer antigen	H44955	0.66
IGHA	IgA	H42228	0.67
FAH	Fumarylacetoacetate hydrolase (fumarylacetoacetase)	H44956	0.67
MMP12	Matrix metalloproteinase 12 (macrophage elastase)	R63637	0.70
OR2A19	Olfactory receptor, family 2, subfamily A, member 19	H39853	0.80
JAG2	Jagged2	H39899	0.84
ESTs	Unknown	H43142	0.84
LIPE	Lipase, hormone sensitive	R87236	0.85
WEE1	Wee 1 like	H44948	0.89
ESTs	Unknown	H29761	0.97
INPP1	Inositol polyphosphate-1-phosphatase	H52141	1.11
DCTN1	Dynactin 1	R88834	1.11
ESTs	Unknown	AI201426	1.13
GATA2	GATA-binding protein 2	R32406	1.39
ESTs	Unknown	H28469	1.65
ESTs	Unknown	H43035	1.72
AP1B1	Adaptor-related protein complex 1, beta 1 subunit	R87770	1.78

were organized on the basis of overall resemblance in their gene expression patterns restricted to these genes (Figure 3). The measure of these similarities was provided by a dendrogram that clearly separated the 3 subgroups of MM. The colored gene expression matrix showed that IgA and IgG transcripts were absent in most of the BJ-MMs, indicating that the lack of IgH protein synthesis observed in these patients was due to either abnormalities at the DNA level or RNA level. A recent study combining Southern

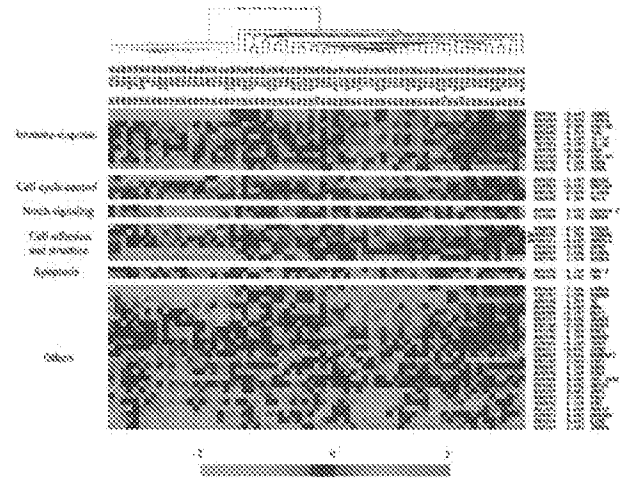

Figure 2. Hierarchic clustering of IgH-secreting MM based on the identified genes (49 members) that best distinguished IgG-MM from IgA-MM. Red-colored branches represent IgA-MM, and black branches represent IgG-MM. Genes were grouped in potential functional categories. Each column represents an MM sample, and each row represents an individual gene. A pseudocolor representation of gene expression is shown according to the scale at the bottom: red indicates expression levels greater than the median, and green indicates levels less than the median. Gene accession numbers, discriminating scores, and gene symbols are labeled on the right. Gray indicates excluded values.

blot and PCR analysis demonstrated that defects at the DNA level are responsible for the lack of IgH protein production in at least 75% of the BJ-MMs studied.[20] Given that 17 of 19 BJ-MM patients we examined have illegitimate rearrangements of the IgH gene (data not shown), our results are also in favor of DNA defects as a major cause of failure to synthesize IgH proteins. Expression level of the other discriminating genes appeared to be globally diminished in almost all BJ-MMs (see colored matrix in Figure 2).

Third, to determine whether IgL subtypes could be phenotypically different, we used discriminating score methods to compare gene expression profiles of 73 patients (13 stage I, 7 stage II, 49 stage III, and 4 PCL). Statistical analysis identified 80 different genes ($P < .0001$) between Igλ- and Igκ-MM (Table 2). As expected, the top-ranked discriminatory genes were Igλ and Igκ. Detailed analysis revealed the presence of several genes essential for bone remodeling, mainly osteoclastogenesis, that were up-regulated in Igκ-MM: *Mip-1α*, *TGFβ3*, and *BMP2*. Conversely, one gene corresponding to a protein that negatively regulates TGFβ activity (*LTBP4*) was overexpressed in Igλ-MM cases.

Because we found several factors known to stimulate osteoclast formation, we wanted to determine whether we could distinguish MM with an increased osteoclast activity from the others. Among the 73 patients previously analyzed, we selected patients with high myeloma cell mass and known score of bone lesions (49 stage III and 4 PCL) and applied hierarchic clustering algorithm according to their expression of the 80 genes that significantly discriminated between Igκ-MM and Igλ-MM. The sample dendrogram distinguished 2 branches (Figure 4A): the left branch grouped 16 of the 21 MMs showing absence or limited lesions (ie, score 0, 1, or 2 according to Durie and Salmon[11] staging system), and the right branch captured 28 of the 32 MMs having multiple bone lesions (ie, score 3 according to Durie and Salmon staging system). Thus, the IgL signature clearly separated MM presenting aggressive bone destruction from the others (χ^2 test, $P < .001$). This gene signature was then tested in a new group of 19 patients (validation group) with stage III MM (6 Igλ-MM and 13 Igκ-MM). The dendrogram (Figure 4B) assigned 5 of 7 MMs without bone disease on the left branch and 11 of 12 MMs with bone disease on the right branch ($P < .001$). This result validates the relationship between bone disease and light chain cluster.

In addition, *Mip-1α* gene expression data obtained with DNA microarray experiments were confirmed by reverse transcriptase–polymerase chain reaction (RT-PCR) analysis (Figure 4C). Interestingly, *IL-1β*, another factor involved in the increase of osteoclast formation and bone destruction in MM,[21] is differentially expressed according to Ig isotype but not according to Ig light chain subtype, supporting the view that IL-1β protein could not be produced by malignant PCs as previously emphasized.[22-24]

Discussion

Gene expression profiling represents a novel molecular approach to examine MM pathogenesis. Using this technology, we addressed questions regarding the marked heterogeneity of MM: do different biologic phenotypes explain divergent clinical courses? Expression profiles of highly purified malignant PCs from 88 MM and 4 PCL patients revealed that specific transcriptional programs are associated with the Ig types and light chain subtypes. Careful analysis of the differentially expressed genes revealed molecular portraits related to disease presentation.

The most discriminating gene between IgG- and IgA-MM, apart from IgH genes, is the transcription factor GATA6, which belongs to the GATA zinc finger family and plays an important role in lung epithelium development.[25] Recently it has been demonstrated that GATA6 regulates WNT7B,[19] a member of the WNT family that is up-regulated in malignant breast tissue[26] and in bladder tumors.[27] Several members of the WNT signaling pathway (WNT5A, WNT10B, FRZB) have been shown to be deregulated in MM.[28,29] Given the key role played by the WNT signaling pathway in carcinogenesis and embryology,[30,31] we can hypothesize that overexpression of these growth factors in MM may play a role in the pathogenesis of MM.

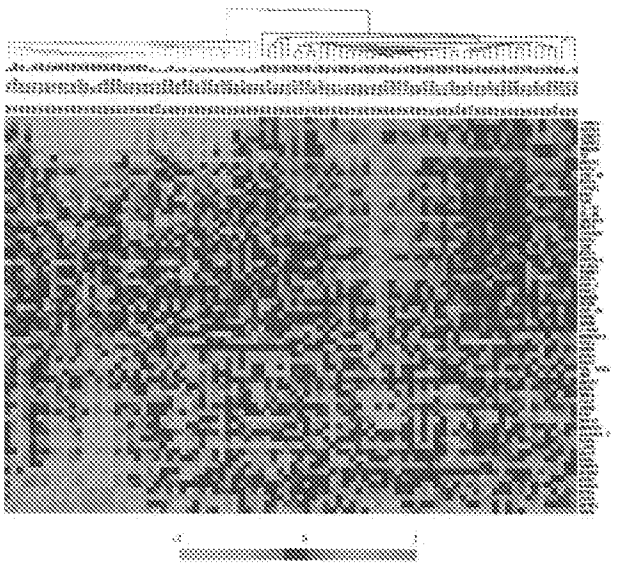

Figure 3. Dendrogram and color matrix representing the hierarchic clustering of the 88 myeloma samples versus 61 genes. The genes used in this analysis were chosen by a discriminating score statistic that is most highly correlated with the 2 IgH isotypes. Blue-colored branches represent BJ-MM, red branches represent IgA-MM, and black branches represent IgG-MM. Columns represent individual MM samples, and rows represent individual genes on the microarray. A pseudocolor representation of gene expression is shown according to the scale at the bottom: red indicates expression levels greater than the median, and green indicates levels less than the median. Gray indicates excluded values.

Table 2. Statistically different genes between Igλ-MM and Igκ-MM

Gene symbol	Gene description	Accession no.	Discriminating score
IGL@	Ig lambda	H14524	−2.74
ESTs	Unknown	H26676	−2.56
ESTs	Unknown	H26661	−1.94
IGL@	Ig lambda	R83196	−1.81
ESTs	Unknown	H15899	−1.80
CDSN	Corneodesmosin	W95594	−1.77
MCP	Membrane cofactor protein (CD46)	H26673	−1.66
ESTs	Unknown	R83001	−1.63
IGL@	Ig lambda	H15030	−1.62
ESTs	Unknown	R74030	−1.33
ABCD4	ATP-binding cassette, subfamily D, member 4	H51632	−1.22
SAMHD1	SAM domain and HD domain, 1	H47862	−1.07
*LTBP4**	Latent transforming growth factor-beta-binding protein 4	R73631	−1.02
ESTs	Unknown	R91051	−1.02
ESTs	Unknown	R89772	−0.99
LMNA	LaminA/C	H26659	−0.98
IGL@	Ig lambda	H41911	−0.95
ESTs	Unknown	R16085	−0.91
ZNF361	Zinc finger protein 361	R73795	−0.90
LTA	Lymphotoxin-alpha	AA910185	−0.81
RPL4	Ribosomal protein L4	N35801	−0.77
ESTs	Unknown	N35710	−0.66
FMO5	Flavin-containing monooxygenase 5	H51750	−0.65
ESTs	Unknown	R72642	−0.64
MXI1	Max-interacting protein 1	AA115514	−0.63
SCN1B	Sodium channel, voltage-gated, type 1, beta subunit	R74526	−0.61
ARHG	Ras homolog gene family, member G	H45512	−0.56
NTE	Neuropathy target esterase	H09751	−0.57
HVEB	Herpesvirus entry mediator B	R73625	−0.56
AXL	AXL receptor tyrosine kinase	H09737	−0.52
GAR22	GAS2-related on chromosome 22	R72509	−0.51
PBX1	Pre-B-cell leukemia transcription factor 1	AA403031	−0.51
HYAL1	Hyaluronoglucosaminidase 1	W84634	−0.50
PRKCI	Protein kinase C, iota form	AA062633	0.50
PLXNB3	Plexin B 3	R41456	0.50
CTSB	Cathepsin B	H98635	0.51
CSNK2A2	Casein kinase II, alpha-2	AA099405	0.53
MMP12	Matrix metalloproteinase 12	R63637	0.54
ACK1	Human activated p21cdc42Hs kinase	R44803	0.54
ESTs	Unknown	AA883518	0.55
EDN3	Endothelin 3	AA553611	0.58
ESTs	Unknown	W74500	0.59
CUL4A	Cullin 4A	AA100650	0.59
DDR1	Discoidin domain receptor family, member 1	AA574033	0.60
*Mip-1α**	Macrophage inflammatory protein 1-alpha (SCYA3)	W74256	0.64
ESTs	Unknown	T86845	0.64
NRID1	Nuclear receptor subfamily 1, group D	R85515	0.66
UBE4A	Ubiquitination factor E4A	R45238	0.66
FGR	Gardner-Rasheed feline sarcoma viral oncogene homolog	W81536	0.66
SIAH2	Seven in absentia, drosophila, homolog of 2	R61665	0.68
ESTs	Unknown	AA548364	0.71
*TGFβ3**	Transforming growth factor, beta-3	W80655	0.73
ZNF198	Zinc finger protein 198	N71655	0.81
TJP2	Tight junction protein 2 (zona occludens 2)	H05082	0.82
CEP3	cdc42 effector protein 3	R43949	0.86
ESTs	Unknown	H89735	0.87
GSTM5	Glutathione S-transferase, mu-5	R40442	0.89
ESTs	Unknown	N58796	0.91
IGKC	Ig kappa	AA284639	0.92
UBE3A	Ubiquitin-protein ligase E3A	AA126792	0.97
DSG1	Desmoglein 1	W72927	0.97
RECQL5	RECQ protein-like 5	R32075	1.04
BPHL	Biphenyl hydrolase-like	AA167197	1.04
ELAVL4	Embryonic lethal, abnormal vision, drosophila, homolog-like 4	R55730	1.06
TNFRSF6	Tumor necrosis factor receptor superfamily, member 6 (CD95)	AA031300	1.06
NR2C1	Nuclear receptor subfamily 2, group C, member 1	H68838	1.10
SMARCA1	Actin-dependent regulator of chromatin, subfamily A, member 1	AA057875	1.12
NFE2L3	Nuclear factor erythroid 2-like 3	R43198	1.17
CYB5	Methemoglobinemia due to deficiency of cytochrome b5	N23249	1.19
LAMB3	Laminin, beta-3	AA622206	1.19
SH3GL2	SH3 domain, GRB2-like 2 (endophilin 1)	R20729	1.20
HSD17B2	17-@beta-hydroxysteroid dehydrogenase II	N23665	1.30
*BMP2**	Bone morphogenetic protein 2	AA114112	1.34
PRKCABP	PRKCA-binding protein	AA641722	1.54
HOX11	Homeobox 11	AA007444	1.61
ESTs	Unknown	N30860	1.66
ESTs	Unknown	AA626328	1.69
EXT1	Exostoses, multiple, type 1	R13402	1.70
ESTs	Unknown	W92915	1.79
IGKC	Ig kappa	R71916	1.82

*Genes known to be involved in bone remodeling.

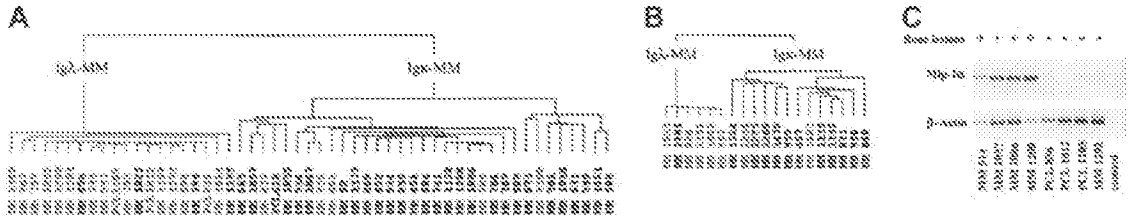

Figure 4. Dendrogam representing the hierarchic clustering of MM patients in stage III and PCL with known bone lesions; score based on the genes of IgλA expression signature. (A) Hierarchic tree of 53 MMs (preliminary group). (B) Hierarchic tree of 19 MMs (validation group). Blue-colored branches represent MMs showing absence or limited bone lesions; black branches represent MMs displaying multiple bone lesions. (C) RT-PCR experiments showing *Mip-1α* mRNA expression in 8 patients, labeled (+) for multiple bone lesions or (−) for absence of or limited bone lesions. Control indicates no template in the PCR reaction.

Analysis of genes included in the immune response cluster is of particular interest. One of the most discriminating genes is *Id2*, a member of the Id class of the helix-loop-helix proteins. Id proteins can act as negative regulators of the class I helix-loop-helix proteins (E proteins), which are known to play a crucial role in lymphocyte development and activity.[32,33] Furthermore, ectopic expression of Id3 in mature activated B cells interferes with IgA expression.[34] These data are in agreement with our results that showed a relative underexpression of *Id2* in IgA-MM. Because the ratio of E proteins to Id proteins appears to be important for later stages of B-cell development, it is important to determine their respective expression in MM. Another gene of interest in this cluster is *IL-16*. Our DNA microarray data showed that *IL-16* was up-regulated in IgG-MM patients. Its expression in purified myeloma cells has been recently reported.[35] IL-16 has been previously described in a variety of cells including $CD4^+$, $CD8^+$ T cells, dendritic cells, and $CD19^+$ B cells.[36,37] IL-16 is a ligand of CD4 and induces in vitro chemotaxis of $CD4^+$ T cells,[38] and CD4 dendritic cells,[36] and $CD8^+$ T cells.[39] Furthermore, IL-16 markedly inhibits CD3-induced T-cell activation, but this effect is limited to $CD4^+$ T cells.[40] Recently, Koike et al[41] have shown that IL-16 serum level of stage III MM was significantly higher than that of normal controls. In addition, the authors found a correlation between IL-16 levels and CD4/CD8 ratio. Taken together, these observations suggest that malignant PCs could be a source of IL-16 and that deregulated expression of IL-16 could contribute to change of the T-cell phenotype in this disease.[42,43]

Among the 3 cell cycle regulators (*c-myb*, *BRCA1*, and *ZNF148/ZBP-89*) overexpressed in IgG-MM, 2 of them, BRCA1 and ZNF148, have been shown to bind to the C terminus of p53 and stabilized specifically the wild-type form.[44-46] Furthermore, overexpression of ZNF148 induced growth arrest and apoptosis.[44] It may be highly interesting to investigate the functional role of BRCA1 and ZNF148 in the pathogenesis of MM. We can speculate that accumulation of wild-type p53 in malignant PCs could increase efficacy to radiotherapy and chemotherapy, explaining in part the better prognosis of IgG-MM compared with IgA-MM.[47,48]

Two genes involved in Notch signaling are overexpressed in IgA-MM samples compared with IgG-MM samples, *GATA2* and *Jagged2*. Maintenance of GATA2 expression is necessary for Notch signaling in hematopoietic cells.[49] Jagged2, the ligand for Notch1,[50] was previously found to be up-regulated in MM cell lines as compared with EBV (Epstein-Barr virus)–immortalized polyclonal B cells derived from the same patient.[29] Because Notch signaling is known to inhibit differentiation of hematopoietic cells,[51] overexpression of *GATA2* and *Jagged2* could favor the maintenance of malignant immature cells in IgA-MM. Another gene overexpressed in IgA-MM, *SRF*, is of interest because it regulates an antiapoptotic member of the Bcl-2 family, Mcl-1.[52] Very recent results from our laboratory and others demonstrated that Mcl-1 is required for the survival of MM cells.[53,54] Conversely, an inducer of apoptosis, *DAP-1*, is down-regulated in the same group of patients. DAP-1 is a member of the ubiquitin-homology proteins that specifically interact with the death domain of TNF-R1 and induce apoptosis in a variety of cell lines.[55] This deregulated balance between proapoptotic and antiapoptotic molecules (in favor of antiapoptotic signals) may explain, at least in part, the lesser chemosensitivity of IgA-MM.

Our analysis of gene expression data of IgH-MM subtypes revealed that genes associated with inhibition of differentiation and apoptosis were up-regulated in IgA-MM, while genes associated with immune response, cell cycle control, and apoptosis induction were down-regulated in this subgroup of MM. These findings were consistent with previous studies demonstrating that the IgA isotype is significantly associated with a shorter survival.[47,48,56] In addition, the fact that BJ-MM patients have strongly diminished IgA and IgG gene signatures suggests that transcriptional programs related to IgH class switch recombination are maintained up to differentiation of activated B cells into PCs in the bone marrow.

The presence of several genes known to be involved in bone remodeling, mainly *Mip-1α*[24,57-59] but also *TGFβ3*,[60-62] *BMP2*,[63] and *LTBP4*,[64] in the cluster of genes differentiating Igκ-MM and IgλA-MM (indicated by asterisks in Table 2) led us to focus our analysis on clinical presentation and IgL subtypes. Using the IgL discriminating cluster of genes to classify the MM patients, we found a strong association between patients lacking bone lesions and IgλA subtype. Our results confirmed previous studies showing that (1) almost all osteosclerotic MM, without or with the paraneoplastic syndrome (POEMS [polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin lesions]), are IgλA-MM[65]; (2) patients unable to destroy bones even at the end stage of the disease are osteoblastic MM at the histologic level and are IgλA-MM[66]; and (3) two thirds of IgλA-MMs reach high cell mass at diagnosis without lytic bone involvement in contrast to Igκ-MM.[66] Of major interest, our DNA microarray and RT-PCR data revealed a correlation between increase of *Mip-1α* mRNA and severity of bone destruction associated with IgκA subtype. Similarly, very recent results reported by Abe et al[59] showed an increase of Mip-1α secretion in highly purified MM plasma cells compared with normal PCs. Given that Mip-1α induced in vitro osteoclast formation in human bone marrow cultures[58] and that highly purified PCs of MM patients with active bone disease induced bone resorption in vitro,[59] the overall data support the view that Mip-1α plays a major role in vivo in MM-induced bone resorption and that osteolytic activity found in IgκA-MM is more likely related to an excessive bone resorption than to a decrease of bone formation. The next step will be the identification of specific transcription factors able to induce Mip-1α expression in IgκA-MM rather than in IgλA-MM. Because Mip-1α promoter contains GATA2 plus AML-1

plus C/EBPα regulatory regions,[67] these transcription factors appear as good candidates to regulate Mip-1α expression in MM.

In conclusion, our attempt to examine heterogeneity of MM by using the DNA microarray approach led us to identify genes whose deregulated expression was associated with the pathogenesis of MM. In addition, our results provide an explanation for the association between Igκ-MM and bone destruction. The major goal of this global approach is to establish a gene expression–based survival predictor for the newly diagnosed patients included within clinical trials of the Intergroupe Francophone du Myélome.

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